## Claims

- 1. A method for regulating the expression of a gene of interest in a host cell that comprises a CodY-like protein comprising providing said cell with a gene of interest in operable linkage with a promoter and at least one CodY target sequence.
- 5 2. A method according to claim 1, wherein said promoter and/or said CodY target sequence is heterologous with regard to said gene of interest.
  - 3. A method according to claim 1 or 2, wherein said CodY target sequence is heterologous with regard to said promoter.
- 4. A method according to any one of claims 1 to 3, wherein said gene of interest is a gene from a gram-positive bacterium.
  - 5. A method according to any one of claims 1 to 4, wherein said gene of interest encodes a protease or a peptidase or an anti-microbial peptide or a vitamin.
- 6. A method according to any one of claims 1 to 5, wherein said CodY
  target sequence comprises a sequence as depicted in Figure 6A or a functional equivalent and/or a functional fragment thereof.
  - 7. A method according to any one of claims 1-6, wherein said CodY target sequence comprises a sequence as depicted in Figure 6B or a functional equivalent and/or a functional fragment thereof.
- 20 8. A method according to any one of claims 1-7, wherein said CodY target sequence comprises a sequence as depicted in Table 4, Table 4A, Table 5, Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a functional fragment thereof.
- A method according to any one of claims 1-8, wherein said CodY target
   sequence comprises an ATGTTCA sequence and an inversely repeated
   ATGTTCA sequence.

- 10. A method according to claim 9, wherein said nucleic acid sequence comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
- 11. A method according to claim 9 or 10, wherein said nucleic acid sequence comprises the sequence ATGTTCAGAAAATTCATGAACAT.
- 12. A method according to any one of claims 1 to 11, further comprising influencing the binding between said CodY-like protein and said at least one CodY target sequence.
- 13. A method according to claim 12, wherein said binding is regulated by subjecting said cell to a change in a growth condition.

- 14. A method according to claim 12 or 13, wherein said binding is regulated by subjecting said cell to a growth limiting condition.
- 15. A method according to claim 14, wherein said growth limiting condition is a limited availability of a nitrogen source.
- 15 16. A method according to any one of claims 1 to 15, wherein said host cell is a cell from a (dairy) food production species.
  - 17. A method according to claim 16, wherein said species is selected from a Lactococcus or Lactobacillus or Streptococcus or Leuconostoc or Pediococcus or Bifidobacterium or Carnobacterium or Propionibacterium.
- 20 18. A method according to any one of claims 1 to 17, wherein said host cell is provided with a nucleic acid encoding a CodY-like protein.
  - 19. An isolated or recombinant nucleic acid that comprises at least one CodY target sequence or a functional fragment and/or a functional equivalent thereof.
- 25 20. A nucleic acid according to claim 19, further comprising a promoter in operable linkage with a gene of interest.
  - 21. A nucleic acid according to claim 19 or 20 further comprising a gene encoding a CodY-like protein.

- 22. A nucleic acid according to claim 20 or 21, wherein said promoter and/or said at least one CodY target sequence is heterologous with regard to said gene of interest.
- 23. A nucleic acid according to any one of claims 20 to 22, wherein said CodY target sequence is heterologous with regard to said promoter.

5

10

- 24. A nucleic acid according to any one of claims 20 to 23, wherein said gene of interest is a gene from a gram-positive bacterium.
- 25. A nucleic acid according to any one of claims 20 to 24, wherein said gene of interest encodes a protease or a peptidase or an anti-microbial peptide or a vitamin.
- 26. A nucleic acid according to any one of claims 19 to 25, wherein said CodY target sequence comprises a sequence as depicted in Figure 6A or a functional equivalent and/or a functional fragment thereof.
- 27. A nucleic acid according to any one of claims 19 to 26, wherein said
   15 CodY target sequence comprises a sequence as depicted in Figure 6B or a functional equivalent and/or a functional fragment thereof.
  - 28. A nucleic acid according to any one of claims 19 to 27, wherein said CodY target sequence comprises a sequence as depicted in Table 4, Table 4A, Table 5, Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a functional fragment thereof.
  - 29. A nucleic acid according to any one of claims 19 to 28, wherein said CodY target sequence comprises an ATGTTCA sequence and an inversely repeated ATGTTCA sequence.
- 30. A nucleic acid according to claim 29, wherein said nucleic acid sequence comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
  - 31. A nucleic acid according to claim 29 or 30, wherein said nucleic acid sequence comprises the sequence ATGTTCAGAAAATTCATGAACAT.
- 32. A vector comprising a nucleic acid according to any one of claims 19 to 30 31.

- 33. A gene delivery vehicle comprising a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32.
- 34. A host cell comprising a nucleic acid according to any one of claims 19 to 31, a vector according to claim 32 or a gene delivery vehicle according to claim 33.
- 35. A host cell according to 34 which is a cell from a (dairy) food production species.
- 36. A host cell according to claim 34 or 35, wherein said species is selected from a *Lactococcus* or *Lactobacillus* or *Streptococcus* or *Leuconostoc* or
- 10 Pediococcus or Bifidobacterium or Carnobacterium or Propionibacterium.

- 37. Use of at least one CodY target sequence for regulating the expression of a gene of interest.
- 38. Use according to claim 37, wherein said target sequence comprises a sequence as depicted in Figure 6A, Figure 6B, Table 4, Table 4A, Table 5,
- Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a functional fragment thereof.
  - 39. Use according to claim 37 or 38, wherein said CodY target sequence comprises an ATGTTCA sequence and an inversely repeated ATGTTCA sequence.
- 20 40. Use according to any one of claims 37 to 39, wherein said nucleic acid sequence comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
  - 41. Use according to any one of claims 37 to 40, wherein said nucleic acid sequence comprises the sequence ATGTTCAGAAAATTCATGAACAT.
- 42. A method for producing a (dairy) food product comprising a step wherein a nucleic acid according to any one of claims 19 to 31, a vector according to claim 32, a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 is used.
- 43. A method according to claim 42, wherein said dairy product is a cheese 30 or a fermented milk product.

WO 2005/003354 PCT/NL2004/000474

- 44. A cheese or a fermented milk product obtainable by a method according to claim 42 or 43.
- 45. A method for at least in part preventing the formation of off-flavours during a process for the production of a (dairy) food product, comprising providing at least one CodY target sequence upstream of a gene which product is, directly or indirectly, involved in the formation of off-flavours.

5

10

15

- 46. Use of a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32 or a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 for increasing the expression of a gene of interest in a stationary phase culture or equivalents of said culture.
- 47. Use according to claim 46, wherein said gene of interest comprises a gene encoding an antimicrobial substance, such as a bacteriocin.
- 48. Use according to claim 46, wherein said gene of interest comprises a gene encoding a flavour compound, vitamin, or a proteinaceous molecule involved in cell lysis.
- 49. Use of a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32 or a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 for decreasing the expression of a gene in a stationary phase culture or equivalents of said culture.
- 20 50. Use according to claim 49, wherein an antisense nucleic acid sequence of an undesired gene is provided in operable linkage with a promoter and at least one CodY target sequence.
  - 51. Use according to claim 49 or 50, wherein said gene is involved with acidification.